

2 **Morphology and ultrastructure of Pavan's gland of**  
3 ***Aneuretus simoni* (Formicidae, Aneuretinae)**

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6 **Keywords:** *Aneuretus simoni*, Aneuretinae, Pavan's gland, morphology, ultrastructure

7 **Running title:** Morphology and ultrastructure of Pavan's gland of *Aneuretus simoni*

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**Abstract**

Workers of *Aneuretus simoni* have a well-developed Pavan's gland, which is the exocrine character that makes the Aneuretinae and Dolichoderinae sister groups. The secretory epithelium of the gland consists in both minor and major workers of an anterior and a posterior part, which makes them different from the Dolichoderinae where the gland is formed by a single part only. At the ultrastructural level, the secretory cells are characterized by a very prominent smooth endoplasmic reticulum, which is in line with the production of trail pheromones in this species. The presence of the gland and hence the ability to lay trail pheromones can be understood as literature data report trail involving behaviours in both castes, minor workers being more active in foraging, while majors play a role during nest emigration.

## Introduction

Ants are known as walking glandular batteries (Hölldobler & Wilson 1990) with an exocrine repertoire in which currently 84 glands are known (Billen & Šobotník 2015). Some glands are known in every ant species, while others are found only in particular groups. An example of the latter situation is Pavan's gland, which is unique for representatives of the Dolichoderinae. The only other species in which a Pavan's gland also occurs is in the aneuretine, *Aneuretus simoni*. This exocrine gland characteristic, together with mainly fossil and molecular data, supports the position of the Aneuretinae as sister group of the Dolichoderinae, which is well recognized in all ant phylogenetic studies (e.g. Brown 1954; Wilson et al. 1956; Taylor 1978; Brady et al. 2006; Ward 2007; Moreau & Bell 2013).

Pavan's gland comprises a glandular epithelium underneath the anterior part of the 7<sup>th</sup> abdominal sternite and a reservoir that is formed by an invagination of the intersegmental membrane between the 6<sup>th</sup> and 7<sup>th</sup> sternites. The reservoir part was first described in workers of *Linepithema* (at that time *Iridomyrmex*) *humile* by Pavan and Ronchetti (1955), who called it the 'organe ventrale' (the ventral organ). The first mention of "Pavan's gland" was made by Wilson (1963), referring again to the reservoir part only. The presence of a conspicuous epithelial gland underneath the anterior part of the 7<sup>th</sup> abdominal sternite was first noticed in the dolichoderines *Conomyrma bicolor*, *Iridomyrmex pruinosus* and *Liometopum apiculatum* as well as in *Aneuretus simoni* by Hölldobler and Engel (1978). It was already known since 1959 that Pavan's gland is the source of the trail substance in dolichoderine ants (Wilson & Pavan 1959), making it one of the first reports on the existence trail pheromones in ants. The trail function was later also confirmed in *A. simoni* by Traniello and Jayasuriya (1981a,b), although the chemical nature of the pheromone in this species is not yet known.

Being the sole extant representative of the subfamily Aneuretinae, *A. simoni* is of special importance for ant phylogeny, and since 1996, due to its presence only in southern Sri Lanka, appears on the IUCN Red List as 'critically endangered'. As more nest sites have recently been reported (Dias & Ruchirani 2014, and references therein), this status was adjusted to 'endangered' (Fellowes & Brühl 2009). We were able to collect a colony fragment of *A. simoni* in 1986, and started a study of its

exocrine system beginning with the intramandibular gland (Billen & Verbesselt 2016). We here report on the morphology and ultrastructure of the Pavan's gland, and compare it with that of the Dolichoderinae.

## Material and methods

Our study material consisted of a small colony fragment with 13 minor and 2 major workers of *A. simoni* that we collected in Gilimale Forest in southern Sri Lanka in December 1986. The posterior part of their abdomen was fixed in 2% glutaraldehyde in a 50 mM Na-cacodylate buffer with 150 mM saccharose and postfixed in 2% osmium tetroxide in the same buffer. Tissues were dehydrated in a graded acetone series, embedded in Araldite and sectioned with a Leica EM UC6 ultramicrotome. Serial semithin sections with a thickness of 1  $\mu\text{m}$  were stained with methylene blue and thionin and examined in an Olympus BX-51 microscope. Thin sections with a thickness of 70 nm were double stained with lead citrate and uranyl acetate and examined in a Zeiss EM900 electron microscope.

## Results

Semithin longitudinal sections through the abdominal tip of both minor (Fig. 1A) and major workers (Fig. 1C) show a well-developed Pavan's gland, that in both castes consists of a bilobed secretory epithelium and a wrinkled reservoir. Both the anterior and posterior part of the secretory epithelium are formed by tall columnar cells with round basally located nuclei. In each part, the apical regions of the cells converge towards a large pale subcuticular space (Fig. 1A-C). The anterior part is mushroom-shaped with gland cells of approx. 25 x 5  $\mu\text{m}$  in minor and 40 x 6  $\mu\text{m}$  in major workers. On cross section, the apical cuticular lining, that forms the dorsal wall of the reservoir duct, is clearly V-shaped (Fig. 1B). The posterior part has a semi-spherical shape, with gland cells of approx. 30 x 4  $\mu\text{m}$  in minor and 25 x 4  $\mu\text{m}$  in major workers. The reservoir appears as a pear-shaped ventral sac (the original 'ventral organ') with a length of approx. 100  $\mu\text{m}$  in both minor and major workers (Fig. 1A,C).

Due to the limited material available, we unfortunately only have ultrastructural data of the anterior part of Pavan's gland, which moreover are limited to minor workers only. A general view of the anterior epithelium shows the slender secretory cells with their basally located round nucleus (Fig. 2A). The apical cell membrane is thrown into irregular microvilli, that cross the large subcuticular space (Fig. 2A,B). In its basal region, the intercellular junctions have a tortuous and slightly swollen appearance (Fig. 2C) with locally ovoid intercellular spaces of around 0.1  $\mu\text{m}$  (Fig. 2D). The secretory cells contain numerous mitochondria and some lamellar inclusions (Fig. 2C,E), but their main characteristic is the well-developed smooth endoplasmic reticulum (Fig. 2E). The wrinkled reservoir wall is lined by a sclerotized cuticle with a thickness of 0.3  $\mu\text{m}$ , that covers a layer of squamous epithelial cells that hardly contain any cytoplasm, except in the region of the nucleus (Fig. 2B,E).

## Discussion

The presence of Pavan's gland in *Aneuretus simoni*, together with its role in producing the trail pheromone (Traniello & Jayasuriya 1981a,b), supports the sister group status of Aneuretinae and Dolichoderinae. The first mention, at least of the reservoir part of the gland (the 'organe ventrale'), in *A. simoni* was already made by Miradoli Zatti and Pavan (1957), followed by the observation of the associated glandular epithelium underneath the 7<sup>th</sup> abdominal sternite by Hölldobler and Engel (1978). A first but superficial structural report of the gland was given by Traniello and Jayasuriya (1981b), providing section images but without further histological details. Our present description of Pavan's gland clearly reveals the existence of two distinct parts in the glandular epithelium, which is different from the single part that has so far been described in the Dolichoderinae (Hölldobler & Engel 1978; Fanfani & Dazzini Valcurone 1984; Billen 1985). Billen (1986) reported that the glandular epithelium in *Linepithema humile* can show two parts on a cross section, but these are similar parts that will eventually fuse together, whereas *Aneuretus* has two different parts in the longitudinal plane. The micrographs published in Traniello and Jayasuriya (1981b) also show the presence of an anterior and posterior epithelium in *A. simoni*, though it was not recognized as such at that time.

The functional meaning of two adjacent epithelial clusters remains unclear, and we do not know whether the production of the trail substance can be attributed to one particular or to both epithelia together. We unfortunately could not study the posterior part with electron microscopy. The most conspicuous characteristic of the secretory cells of the anterior part is the abundant smooth endoplasmic reticulum, which can be well understood by the elaboration of a non-proteinaceous pheromonal secretion (Noirot & Quennedey 1974; Billen & Morgan 1998), and which is also common for Pavan's gland of Dolichoderinae (Billen 1985, 1986). The apical microvilli are typical for class-1 epithelial gland cells (Noirot & Quennedey 1974), and allow efficient secretion through their increase of the exchange surface with the exterior. The occurrence of lamellar inclusions is a common cytoplasmic feature in exocrine glands, and corresponds with lipidic secretion (Boudreau et al. 1983). A remarkable feature of Pavan's gland is the structural separation of the secretory cells and the reservoir sac, which requires a special mechanism for the secretory products to find their way to the reservoir. The unusual beak-shape of the reservoir duct at the junction of the 6<sup>th</sup> and 7<sup>th</sup> sternites (Wilson & Pavan 1959) may play a role in this.

The presence of Pavan's gland in both minor and major workers indicates that both castes are able to lay chemical trails. This can be understood by the division of labour in *A. simoni*, for which Traniello and Jayasuriya (1985) reported that foraging, and hence also trail laying, is mainly the role of minors. The few majors in the colony, on the other hand, play a trophic role by regurgitating, but also show a high participation during nest emigrations (Traniello & Jayasuriya 1985), which is a process that also requires trail pheromone communication.

### Acknowledgements

We are very grateful to An Vandoren for her help in section preparation and to Lakshman Weeratunge and Athula Attygalle for their assistance in collecting the ants. We also thank Adam Cronin, José Serrão and Sze Huei Yek for valuable comments on the manuscript.

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## Figure legends

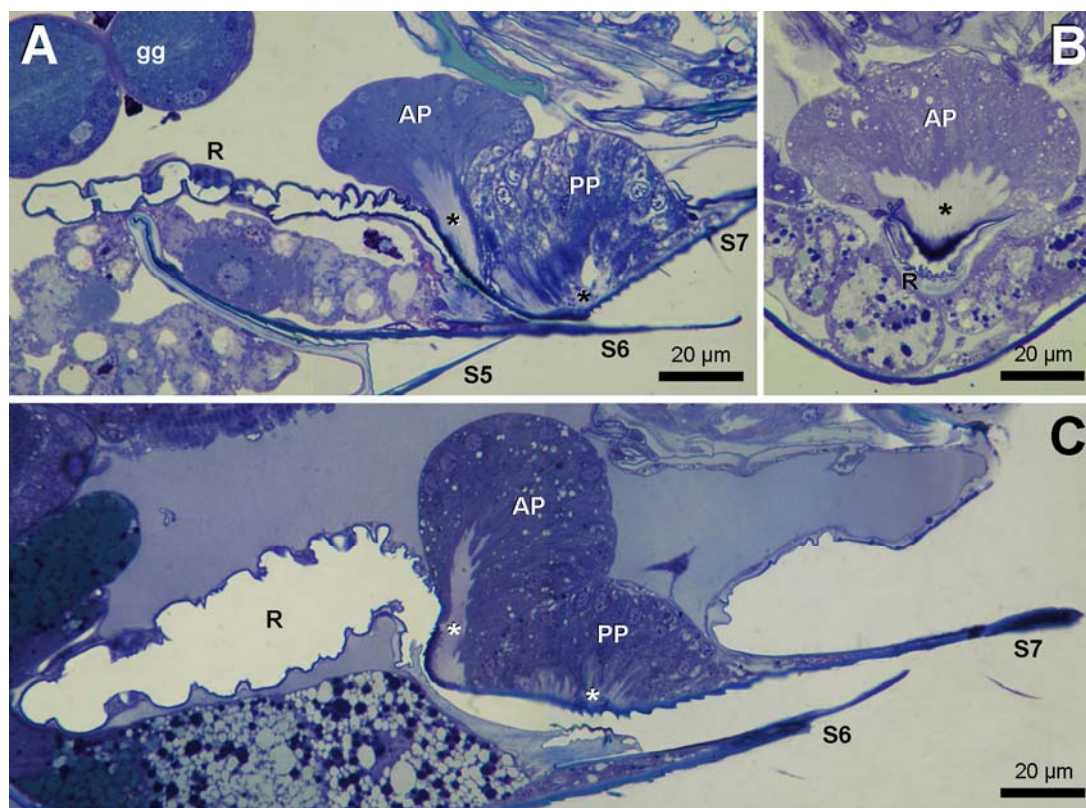


Fig. 1 – **A.** Longitudinal section through ventral part of abdomen tip of minor worker, showing bilobed secretory part of Pavan's gland (AP: anterior part, PP: posterior part, R: reservoir). **B.** Cross section through anterior part of Pavan's gland secretory epithelium in minor worker. **C.** Longitudinal section through Pavan's gland in major worker, also with bilobed appearance. gg: ganglion, S5,6,7: sternites 5,6,7. Asterisks show subcuticular space. All figures shown at same magnification.

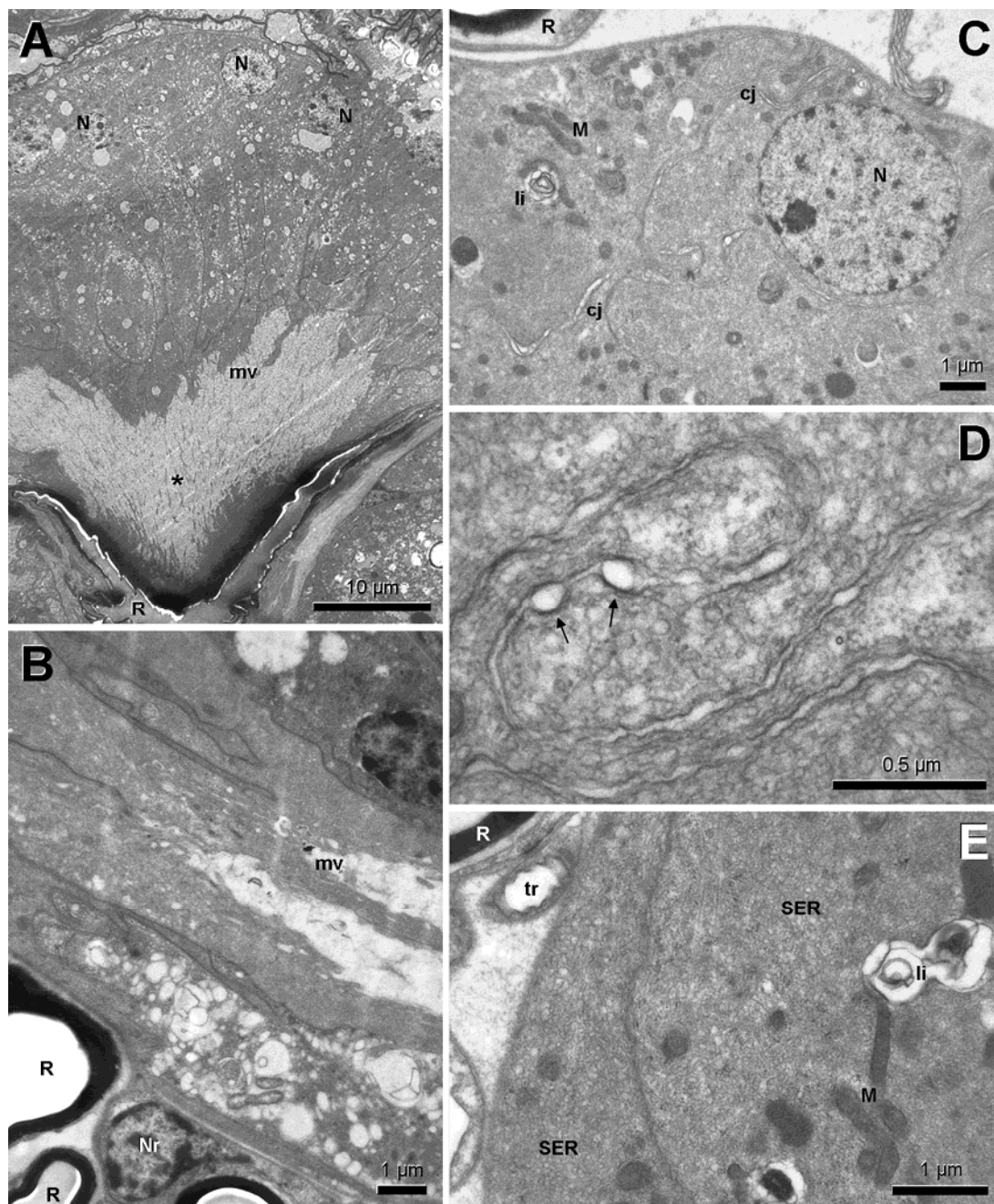


Fig. 2 – Electron micrographs of anterior part of Pavan's gland in minor worker. **A.** Low magnification view in cross section showing columnar cells with basally located nuclei (N), irregular apical border and large subcuticular space (asterisk). **B.** Apical cytoplasm with irregular microvilli (mv) and reservoir wall (R; Nr: nucleus of reservoir wall cell). **C.** Basal cytoplasm with round nuclei, lamellar inclusions (li), numerous mitochondria (M) and contorted cell junctions (cj). **D.** Detail of contorted cell junction in lower epithelial region with locally round intercellular spaces (arrows). **E.** Detail of basal cytoplasm with well-developed smooth endoplasmic reticulum (SER) and numerous mitochondria. tr: tracheole.